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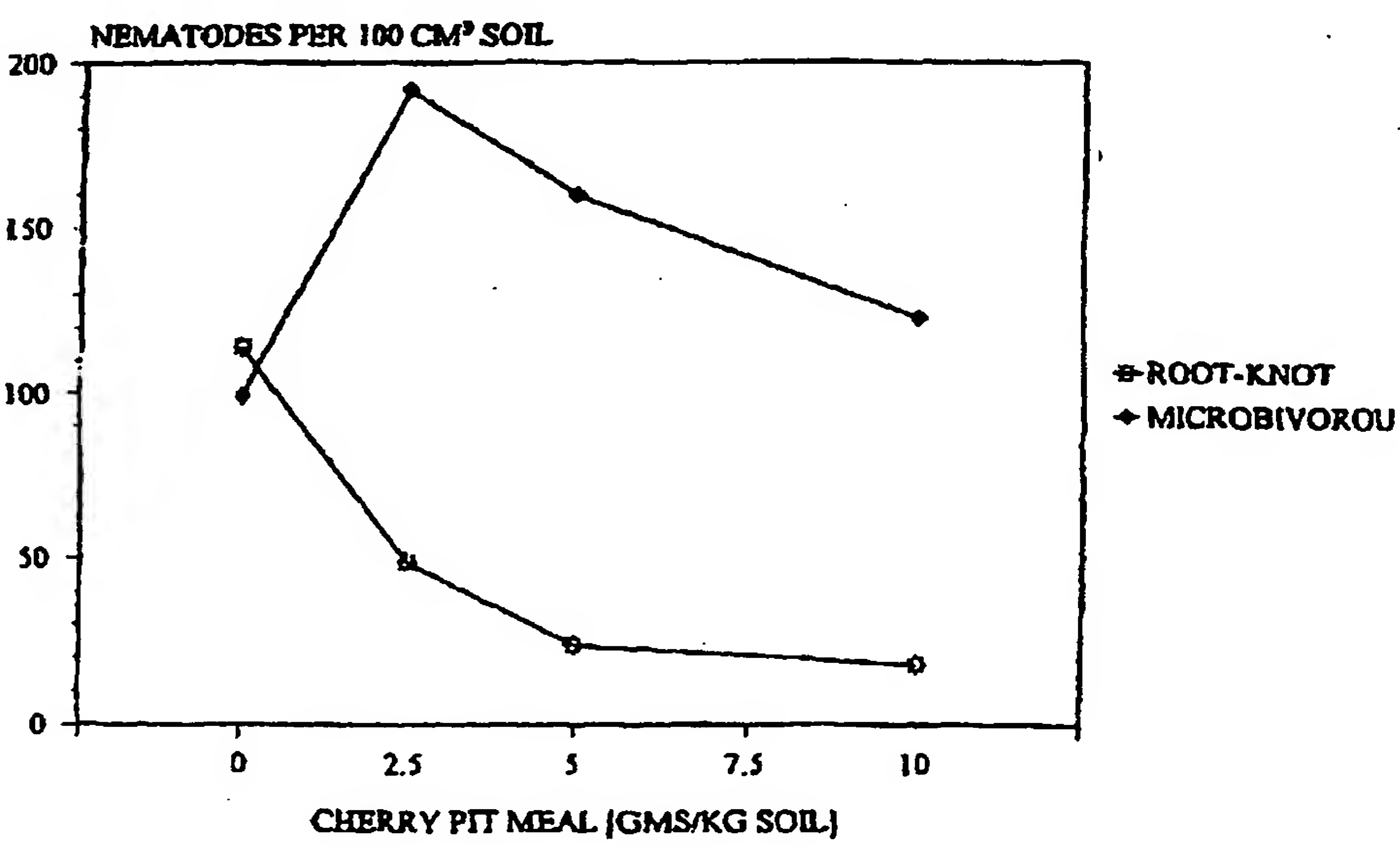
(54) Title: THE USE OF DRUPES AS A SOIL AMENDMENT TO CONTROL NEMATODES



(57) Abstract: A method for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium, and an inexpensive, non-phytotoxic nematicidal composition that can be substituted for environmentally hazardous and toxic synthetic nematicides is disclosed. The composition comprises the ground seeds from a drupe of the*Rosaceae*<> family (e.g., cherry, peach, apple, pear, quince, almond, plum, and apricot), or an extract of these ground seeds. The composition may also include a metal such as soybean meal, and urea or a source of urea. The composition is applied to a plant growth medium in at least nematistically and fungistatically effective amount in order to suppress the growth of phytopathogenic nematodes and fungi in the plant growth medium.

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The Use Of Drupes As A Soil Amendment To Control Nematodes

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States Provisional Patent Application No. 60/293,587 filed May 24, 2001.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with United States government support under contract numbers U.S. EPA 828670-01, U.S. EPA 825532-01, and USDA 00-34189-9045. The United States has certain rights in this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0003] This invention relates to an organic soil amendment derived from the pit material of drupes, or stone fruit. These fruits include, but are not limited to, cherries, peaches, apricots, plums, apples, pears, almond, and quince. The amendment, when applied to soils of growing plants, is effective in controlling pathogenic nematodes and other pests, and in stimulating the beneficial microflora of the rhizosphere.

2. Description of the Related Art

[0004] Nematodes are slender, worm-like organisms found in the soil almost anywhere in the world. Some nematodes are plant parasitic, and hundreds of species of nematodes are known to be plant-pathogenic. Many plants are affected by nematodes, including soybeans, peanuts, cotton, tobacco, strawberries, root crops, ornamentals, citrus, vegetables and many other crops. Nematodes feed on the roots and lower stems of plants, and some attack the leaves and flowers. Some species of nematodes inadvertently introduce pathogenic, root-invading microorganisms into the plants while feeding. Nematodes may also predispose plant varieties to other disease causing agents, such as wilts and root rots. In other instances, the nematodes themselves cause the disease, disrupting the flow of water and nutrients in the xylem system, resulting in root-knot or deprivation of the above-ground parts, and ultimately causing stunting. Symptoms of nematode infestation include swellings, thickenings, galls and distortions of above-ground components of the plant, and

root conditions such as short stubby roots, lesions (dead spots), swellings, galls and general breakdown.

[0005] The extent of crop loss to nematode infestation is substantial and widespread. As a result, various chemical controls to limit the damage caused by nematodes and to curb their spread to uninfested fields have been proposed. Numerous nematicides have been available, some of which are useful as fumigants. The most effective and widely used control agents have been methyl bromide and ethylene dibromide, and certain chlorinated compounds (e.g., 1,3 dichloropropene). Other nematicides generally fall within three groups: (1) organophosphate insecticides, (2) isothiocyanates, and (3) carbamate or oxime insecticides. For example, Vapam™ (sodium N-methyl dithiocarbamate) has proven to be useful, especially as a pre-plant treatment.

[0006] Probably the most effective substance for soil treatment has been methyl bromide. When used as a soil treatment, methyl bromide is injected into the soil to sterilize the soil and kill the vast majority of soil organisms. After the methyl bromide is injected, the soil is covered with plastic tarps that hold most of the methyl bromide in the soil. The tarps are removed 24 to 72 hours later. After the tarps are removed, much of the methyl bromide leaves the soil. It has been reported that the United States Environmental Protection Agency estimates that about 50% to 95% of the methyl bromide in the soil eventually enters the atmosphere where it may damage the ozone layer. Because of the damaging effects of methyl bromide on the ozone layer, the production and importation of methyl bromide in the United States has been prohibited. Other nations have also banned the use of methyl bromide. Similarly, other nematicides have come under increased regulation.

[0007] The removal of key nematicides, such as methyl bromide, from use in several industrialized countries through regulatory action have renewed interest in alternative nematode management methods. Accordingly, non-chemical or organic soil amendments are needed to meet the demand for control of nematodes and other crop pests.

[0008] Several new alternative nematicides have recently been introduced with limited success. These include the toxin from *Myrothecium* marketed by Abbot

Laboratories as Di-Tera™; and ClandoSan™, an organic amendment that includes chitinous material that has been marketed for over 20 years. Others are Safe-T-Green™ 18, a blend of secondary alcohols reacted with ethylene oxide; DENY™ liquid, a suspension of *Burkholderia cepacia* (a bacterium); and Neotrol™ (or Nemotrol™) a product composed of ground sesame plants. None of the alternative nematicides are as broadly effective as the chemical pesticides.

[0009] Other alternative insect or nematode control and/or management methods are reported in the patent literature. For example, U.S. Patent No. 434,243 discloses a composition consisting of ground tobacco plant parts, bran of any type of grains, cotton-seed meal, and insect poison. The composition is applied on top of the ground to nourish a plant's growth and control worms or other insects. U.S. Patent Nos. 5,204,100 and 5,506,099 disclose several *Bacillus thuringiensis* strains that can be used to control *Coleopteran* insects. U.S. Patent No. 5,464,618 discloses a gustatory stimulant composition comprising a dried and powdered *Cucurbitaceae* plant material, a lubricant and an adherent. Optionally, an adulticide may be added to the composition. The composition can be used to control a beetle population of the family *Galerucinae*.

[0010] U.S. Patent No. 6,051,233 discloses a chemical composition comprising heat components, mustard oil, lemon extract, vegetable oil, and surfactants to treat soil to control most diseases and pests. The heat components provide the disease and pest control activity. The heat components may comprise capsaicin, ginger oil, black pepper oil, ginger oleo resin, black pepper oleo resin, capaicinoids, cassia oil, cinnamon leaf oil, cinnamon bark oil, cinnamic aldehyde, bitter almond oil, and benzaldehyde. U.S. Patent No. 4,442,092 discloses a nematicidal composition containing as an active ingredient(s) therein sesame plant extracts including roots, seeds or stalks, oils and acids extracted therefrom. U.S. Patent No. 5,057,141 describes compositions including soybean meal, urea and chitin-containing formulations. These compositions are reported to be non-phytotoxic and to provide cost-effective biological suppression of plant pathogenic nematode populations and excellent plant nutrition. U.S. Patent Nos. 5,882,851 and 6,133,417 disclose P-450 dependent monooxygenases (converting amino acids to cyanohydrins, the precursor of the cyanogenic glycolysis, or to

glucosinolates) and using the monooxygenases to make transgenic plants that are nematode resistant.

[0011] Other articles on nematode control and/or management methods are reported in the scientific literature. See, for example, Bauske, *et al.*, "Effect of botanical aromatic compounds and seed-surface pH on growth and colonization of cotton plant growth-promoting rhizobacteria", *Biocontrol Science and Technology* 7: 415-421, 1997; Kloepper, *et al.*, "Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases", *Australasian Plant Pathology* 28: 21-26, 1999; Mian, et al., "Soil amendments with oil cakes and chicken litter for control of *Meloidogyne arenaria*", Nematropica 12: 205-220, 1982; Mian, *et al.*, "Survey of the nematicidal of some organic materials available in Alabama as amendments to soil for control of *Meloidogyne arenaria*", *Nematropica* 12: 235-246, 1982; Rodriguez-Kabana *et al.*, "Chitinous materials from blue crab for control of root-knot nematode. I. Effect of urea and enzymatic studies", Nematropica 19(1): 53-74, 1989; and Rodriguez-Kabana *et al.*, "Chitinous materials from blue crab for control of root-knot nematode. II. Effect of soybean meal", Nematropica 20(2): 153-168, 1989.

[0012] Therefore, there is a continuing need for inexpensive, non-phytotoxic nematicidal compositions that can be substituted for environmentally hazardous and toxic synthetic nematicides. There is also a need for nematicidal compositions and methods for reclaiming and managing nematode-infested soils, for stimulating the growth of plants in nematode-infested soils, and for imparting sustained nematicidal and fertilizer activity to the soils. There is another need for new soil amendments for the control of phytopathogenic nematodes, and methods for producing the soil amendments. There is a further need for compositions which are obtained from organic sources and which stimulate the reproduction and development in soil of normal microflora which are destructive to plant-pathogenic nematodes.

SUMMARY OF THE INVENTION

[0013] The foregoing needs are met by a method according to the invention for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium. The method comprises applying to the plant growth medium the ground

seeds from a drupe of the *Rosaceae* family. Preferably, the seeds that are ground are obtained from the group consisting of cherries, peaches, apples, pears, quince, almonds, plums, and apricots. Most preferably, the seeds that are ground are obtained from any plant of the genus *Prunus* of the *Rosaceae* family, and the pits of tart or maraschino cherries are particularly preferred. Optionally, the method also includes the step of applying to the plant growth medium a meal other than the ground seeds. Preferably, the meal is selected from the group consisting of soybean meal, cottonseed meal, sunflower seed meal, linseed meal, peanut meal, safflower meal, corn meal, jojoba meal, and sesame meal, and most preferably, the meal is soybean meal. Optionally, the method also includes the step of applying to the plant growth medium urea or a source of urea.

[0014] In another aspect, the invention is a method that comprises applying to the plant growth medium an extract from the ground seeds from a drupe of the *Rosaceae* family. Preferably, the seeds that are ground and extracted are obtained from the group consisting of cherries, peaches, apples, pears, quince, almonds, plums, and apricots. Most preferably, the seeds that are ground and extracted are obtained from any plant of the genus *Prunus* of the *Rosaceae* family, and the pits of tart or maraschino cherries are particularly preferred.

[0015] In still another aspect, the invention is a method that comprises applying to the plant growth medium a material including amygdalin, such as ground seeds from a drupe of the *Rosaceae* family or an extract from the ground seeds from a drupe of the *Rosaceae* family.

[0016] In yet another aspect, the invention is a composition for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium. The composition comprises ground seeds from a drupe of the *Rosaceae* family and a meal selected from the group consisting of soybean meal, cottonseed meal, sunflower seed meal, linseed meal, peanut meal, safflower meal, corn meal, jojoba meal, and sesame meal. Preferably, the seeds that are ground are obtained from the group consisting of cherries, peaches, apples, pears, quince, almonds, plums, and apricots. Most preferably, the seeds that are ground are obtained from any plant of the genus *Prunus* of the *Rosaceae* family. Preferably, the meal in the composition is soybean meal. The composition may be admixed

with soil in order to suppress the growth of phytopathogenic nematodes and fungi in the soil, reduce the amount of phytopathogenic nematodes and fungi in the soil, or kill the phytopathogenic nematodes and fungi in the soil.

[0017] Therefore, it is an advantage of the present invention to provide an inexpensive, non-phytotoxic nematicidal composition that can be substituted for environmentally hazardous and toxic synthetic nematicides.

[0018] It is another advantage of the present invention to provide nematicidal compositions and methods for reclaiming and managing nematode-infested soils, for stimulating the growth of plants in nematode-infested soils, and for imparting sustained nematicidal and fertilizer activity to the soils.

[0019] It is yet another advantage of the present invention to provide soil amendments for the control of phytopathogenic nematodes, and methods for producing the soil amendments.

[0020] It is still another advantage of the present invention to provide compositions which are obtained from organic sources and which stimulate the reproduction and development in soil of normal microflora which are destructive to plant-pathogenic nematodes.

[0021] These and other features and advantages of the invention will become better understood upon consideration of the following detailed description and appended claims.

DETAILED DESCRIPTION OF THE INVENTION

[0022] The present invention provides nematicidal compositions and methods for reclaiming and managing nematode-infested soils, for stimulating the growth of plants in nematode-infested soils, and for imparting sustained nematicidal and fertilizer activity to the soils. A wide variety of plant pathogenic nematodes can be controlled in accordance with the present invention. Non-limiting examples of such nematodes include root-knot (*Meloidogyne incognita*), lance (*Hoploaimus galeatus*), stunt (*Tylenchorhynchus claytoni*), and stubby root (*Patrichodorus minor*) nematodes. Phytopathogenic fungi can also be controlled in accordance with the present invention.

[0023] In a first embodiment, the invention provides a method for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium.

The method comprises applying to the plant growth medium at least a nematistatically and fungistatically effective amount of ground seeds from a drupe of the *Rosaceae* family. By "nematistatically and fungistatically effective amount", we mean at least the minimum amount that suppresses the growth of phytopathogenic nematodes and fungi in a plant growth medium when that amount is added to the plant growth medium. Preferably, the seeds that are ground are obtained from the group consisting of cherries, peaches, apples, pears, quince, almonds, plums, and apricots. Most preferably, the seeds that are ground are obtained from any plant of the genus *Prunus* of the *Rosaceae* family. The pits of tart or maraschino cherries are particularly preferred, and it is preferred that the ground seeds pass a 0.1 millimeter sieve.

[0024] The method for suppressing the growth of phytopathogenic nematodes and fungi can be used in any plant growth medium. Preferably, the plant growth medium is soil. However, the method can also be used in hydroponic horticulture wherein the plant growth medium is a plant growth solution. In one version of the first embodiment of the invention, the plant growth medium is soil and the amount of ground seeds used in the method is at least 0.5 grams per kilogram of soil. In another version of the first embodiment of the invention, the plant growth medium is soil and the amount of ground seeds is about 0.5 to about 10 grams per kilogram of soil. At higher levels of the ground seeds, the amount of phytopathogenic nematodes and fungi in the soil will be reduced, and at even higher levels, the phytopathogenic nematodes and fungi will be killed.

[0025] Optionally, the first embodiment of the invention also includes the step of applying to the plant growth medium a meal other than the ground seeds. The meal may be added separately or with the ground seeds to the plant growth medium. Preferably, the meal is selected from the group consisting of soybean meal, cottonseed meal, sunflower seed meal, linseed meal, peanut meal, safflower meal, corn meal, jojoba meal, and sesame meal. Most preferably, the meal is soybean meal, and the weight ratio of ground seeds to soybean meal is from 0.25 to 5. The same weight ratios are suitable when other meal used. The amount of ground seeds and meal used in the method is preferably at least 0.5 grams of the mixture of ground seeds and meal per kilogram of soil, and most

preferably, the amount of the mixture of ground seeds and meal is about 0.5 to about 20 grams per kilogram of soil.

[0026] Optionally, the first embodiment of the invention also includes the step of applying to the plant growth medium urea or a source of urea (e.g., isobutyldienediurea, crotonylidiene diurea, difurforylidene triureide, oxalyldiuride, biuret and triuret). The urea or a source of urea may be added separately or with the ground seeds to the plant growth medium. Preferably, the weight ratio of the urea or a source of urea to the ground seeds is from 0.01 to 1.

[0027] In a second embodiment, the invention provides another method for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium. This method also stimulates the growth of saprophagous nematodes. The method comprises applying to the plant growth medium at least a nematistatically and fungistatically effective amount of an extract of seeds from a drupe of the *Rosaceae* family. Preferably, the seeds that are extracted are obtained from the group consisting of cherries, peaches, apples, pears, quince, almonds, plums, and apricots. Most preferably, the seeds that are extracted are obtained from any plant of the genus *Prunus* of the *Rosaceae* family. The extract of pits of tart or maraschino cherries is particularly preferred.

[0028] The extract may be prepared by grinding the seeds and extracting the seeds with a solvent. Preferably, the solvent is selected from the group consisting of ethanolamine, hexane, chloroform, supercritical carbon dioxide, propane, liquid nitrogen, and mixtures thereof. In one form of the extraction process, the seeds are from any plant of the genus *Prunus* of the *Rosaceae* family, the solvent is ethanolamine, and a mixture of the seeds and the solvent is heated during extraction to temperatures of 20°C to 75°C. In another form of the extraction process, the seeds are from any plant of the genus *Prunus* of the *Rosaceae* family, the solvent is hexane, and a mixture of the seeds and the solvent is extracted using soxhlet extraction.

[0029] This method for suppressing the growth of phytopathogenic nematodes and fungi can also be used in any plant growth medium. Preferably, the plant growth medium is soil. However, the method can also be used in hydroponic horticulture wherein the plant growth medium is a plant growth solution. In one

version of the second embodiment of the invention, the plant growth medium is soil and the amount of extract added to the soil is at least 0.5 milliliters per kilogram of soil. In another version of the second embodiment of the invention, the plant growth medium is soil and the amount of extract added to the soil is about 0.5 to about 10 milliliters per kilogram of soil. At higher levels of the extract, the amount of phytopathogenic nematodes and fungi in the soil will be reduced, and at even higher levels, the phytopathogenic nematodes and fungi will be killed.

[0030] In a third embodiment, the invention provides a composition for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium. The composition comprises ground seeds from a drupe of the *Rosaceae* family and a meal selected from the group consisting of soybean meal, cottonseed meal, sunflower seed meal, linseed meal, peanut meal, safflower meal, corn meal, jojoba meal, and sesame meal. Preferably, the seeds that are ground are obtained from the group consisting of cherries, peaches, apples, pears, quince, almonds, plums, and apricots. Most preferably, the seeds that are ground are obtained from any plant of the genus *Prunus* of the *Rosaceae* family. The pits of tart or maraschino cherries are particularly preferred, and it is preferred that the ground seeds pass a 0.1 millimeter sieve.

[0031] Preferably, the meal in the composition is soybean meal, and the weight ratio of ground seeds to soybean meal is from 0.25 to 5. The same weight ratios are also suitable when other meal used. The composition is particularly effective in suppressing the growth of phytopathogenic nematodes and fungi in soil when at least 0.5 grams of the composition is admixed per kilogram of soil. Most preferably, the amount of the composition admixed with soil is about 0.5 to about 20 grams per kilogram of soil. Optionally, the composition of the third embodiment of the invention also includes urea or a source of urea (e.g., isobutyldienediurea, crotonylidiene diurea, difurforylidene triureide, oxalyldiuride, biuret and triuret). Preferably, the weight ratio of the urea or a source of urea to the ground seeds is from 0.01 to 6.

[0032] In a fourth embodiment, the invention provides another method for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium. The method comprises applying to a plant growth medium at least a

nematistatically and fungistatically effective amount of a material including amygdalin. In one version of the fourth embodiment, the material is a ground solid material such as ground seeds from a drupe of the *Rosaceae* family. In another version of the fourth embodiment, the material is a solution including amygdalin such as an extract from the seeds from a drupe of the *Rosaceae* family. Preferably, the amygdalin is present in the material in an amount of at least 100 ppm of the total weight of the material, and most preferably, the amygdalin is present in the material in an amount of 100 ppm to 30,000 ppm of the total weight of the material. When the material is a solution, amygdalin levels of 1,000 to 10,000 ppm by total weight of the solution are quite useful.

[0033] Plants susceptible to nematode infestation which can be aided with the present invention include but are not limited to field crops, such as tobacco, peanuts, rice and cotton; fruit and nut crops, such as strawberries, cranberries, dates, pineapples, olives, coffee and tea; citrus fruits including oranges, tangerines, grapefruit, lemons, and limes; deciduous fruits including apples, apricots, bananas, cherries, grapes, nectarines, kiwi fruit, peaches, pears, plums and prunes; and nuts including almonds, filberts, macadamias, pecans, pistachios and walnuts; commercial vegetable and melon crops, such as celery, eggplant, lettuce, tomatoes, peppers, cauliflower, onions, carrots, broccoli, honeydew melons, white potatoes, and sweet potatoes; and floral crops, lawns and turf, and ornamentals, including cut flowers, flowering pot plants and bedding and foliage plants, including orchids, chrysanthemums, begonias, gardenias, poinsettias, boxwood and ferns, and turfgrasses for golf courses, cemeteries, office and industrial parks, recreational parks, and lawns.

[0034] Because of the enhanced nematicidal activity and fertilizer effect that is induced by combining soybean meal (and optionally urea) with the nematicidally active ground seeds from a drupe of the *Rosaceae* family, addition to soils of compositions including these two components provides an economically and environmentally attractive means for reducing food, fiber and economic losses due to nematode infestations and a means for the use of otherwise low value seeds from a drupe of the *Rosaceae* family.

Examples

[0035] The following Examples have been presented in order to further illustrate the invention and are not intended to limit the invention in any way. Example 1 describes greenhouse experiments that were performed to determine the nematicidal activities of cherry pit meal and mixtures of cherry pit meal and soybean meal on growing soybean plants. Example 2 describes greenhouse experiments that were performed to determine the nematicidal activities of different cherry pit meals and mixtures of different cherry pit meals and soybean meal on growing tomato plants. Example 3 describes greenhouse experiments that were performed to determine the nematicidal activities of cherry pit extract on growing tomato and squash plants.

Example 1

[0036] As an initial experiment, ground cherry pits were applied to nematode-containing soil at rates of 0, 2.5, 5, and 10 grams per kilogram of soil. The soil amendment was effective in killing nematodes including *Meloidogyne arenaria* (root-knot nematode) and also stimulating beneficial microflora of the rhizosphere. The results are shown in Figure 1.

[0037] Soil was then obtained from a cotton field infested with root-knot (*Meloidogyne incognita*), lance (*Hoploaimus galeatus*), stunt (*Tylenchorhynchus claytoni*), and stubby root (*Patrichodorus minor*) nematodes as well as phytopathogenic fungi. The soil was mixed 1:1 (v:v) with fine (<1 mm.) washed siliceous river sand and the mixture was apportioned in 1 kilogram amounts into 4 liter capacity plastic bags. This soil-sand mixture will be referred to hereinafter in Example 1 and Table 1 as soil.

[0038] Cherry pit meal was prepared by grinding whole cherry pits to pass a 0.1 millimeter sieve. Twelve soil mixtures were then prepared as shown in the following Table 1 using the 1 kilogram amounts of soil in the 4 liter capacity plastic bags. The test materials were added to the bags and mixed thoroughly with the moist (60% field capacity) soil and transferred to 1 liter capacity 4-inch diameter cylindrical plastic pots.

[0039] It can be seen from Table 1 that cherry pit meal was added to the soil at rates of 0, 2, 4, and 6 grams per kilogram of soil. These cherry pit meal

treatments were added alone and in combination with 2 and 4 grams of commercially available soybean meal per kilogram of soil. Thus, there were a total of 12 treatments in the experiment of Example 1. The 12 treatments represented all rates of soybean meal and cherry pit meal alone and all possible combinations of soybean meal and cherry pit meal rates. Each rate and controls were represented by eight replications (experimental unit = one pot) arranged in a randomized complete block design in a greenhouse. A no-treatment control and a positive control consisting of 10 milliliters of 10% metam sodium (Vapam™), a broad-spectrum fumigant, were included in each experiment.

TABLE 1

Mixture Number (8 replications)	Soil (kg.)	Cherry Pit Meal (grams)	Soybean Meal (grams)	Vapam™ 10% sodium N-methyl dithiocarbamate (ml.)
No Treatment Control	1	0	0	-
Positive Control	1	0	0	10
1-1	1	0	0	-
1-2	1	2	0	-
1-3	1	4	0	-
1-4	1	6	0	-
1-5	1	0	2	-
1-6	1	2	2	-
1-7	1	4	2	-
1-8	1	6	2	-
1-9	1	0	4	-
1-10	1	2	4	-
1-11	1	4	4	-
1-12	1	6	4	-

[0040] The pots were placed on a greenhouse table and were kept moist for 10 days when soil samples were collected from each pot for nematological analysis using methods described in Rodriquez-Kabana *et al.*, "A simple method for extraction nematodes from the soil", *Nematropica* 11: 175-185, 1981. The pots were then planted (5 seeds/pot) with "Young" soybean (Glycine max). The ensuing plants were kept in good growing conditions for eight weeks when the plants were removed and soil samples collected for nematological analysis. The

roots were examined for damage, the number of root galls caused by *M. incognita* were counted, and the weights of fresh roots and shoots recorded. The general appearance of the roots was assessed using a subjective index scale where 5 represented the worst looking roots and 1 those with a healthy appearance. Root condition was used as an indication of resistance to both nematodes and phytopathogenic fungi. The roots were incubated for 72 hours to determine nematode populations in their tissues using the same method as for the soil samples. All data were analyzed by standard procedures for analysis of variance and Fisher's Least Significant Differences were calculated ($P = 0.05$) when F values were significant. All differences among means referred to herein were significant at $P \leq 0.05$.

[0041] Data from analyses of preplant and final soil samples indicated that cherry pit meal suppressed root-knot, lance, and stunt nematodes at rates > 2 grams/kilogram of soil. At 6 grams/kilogram of soil the cherry pit meal reduced Root-knot nematode populations by 88.5%. At 6 grams/kilogram of soil cherry pit meal plus 2 grams/kilogram of soil soybean meal Root-knot and Stunt nematodes were completely eliminated and Lance nematodes were reduced >99%. Shoot weight was increased >40% over controls and overall root condition was excellent. The addition of soybean meal increased nematicidal activity considerably and made performance of the amendments much more consistent (less variation among replications). The number of beneficial microbivorous nematodes (mostly *Rhabditidae*) increased with increasing amounts of amendment added to soil and was definitely stimulated (>25% increase) by mixing cherry pit meal with soybean meal. Changes in root populations of plant parasitic nematodes followed the pattern described for soil populations, i.e. increased suppression in response to cherry pit meal rates and cherry pit meal + soybean meal mixtures more effective than cherry pit meal alone.

[0042] Without intending to be bound by theory, it is believed that the mechanism of nematicidal activity of cherry pits (and other similar seeds from a drupe of the *Rosaceae* family) involves the breakdown of the glycoside amygdalin by the enzyme β-glucosidase, releasing hydrogen cyanide and benzaldehyde. It is believed that any stone fruit (drupe) or other seed containing amygdalin,

including, but not limited to, peaches, plums, apricots, apples, pears, and quince, will show efficacy against nematodes and other crop pests.

Example 2

[0043] Soil was obtained from a cotton field infested with root-knot (*Meloidogyne incognita*), lance (*Hoploaimus galeatus*), stunt (*Tylenchorhynchus claytoni*), and stubby root (*Patrichodorus minor*) nematodes as well as phytopathogenic fungi. The soil was mixed 1:1 (v:v) with fine (< 1 mm.) washed siliceous river sand and the mixture was apportioned in 1 kilogram amounts into 4 liter capacity plastic bags. This soil-sand mixture will be referred to hereinafter in Example 2 and Table 2 as soil.

[0044] Maraschino cherry pit meal was prepared by grinding whole maraschino cherry pits to pass a 0.1 millimeter sieve. Tart cherry pit meal was also prepared by grinding whole tart cherry pits to pass a 0.1 millimeter sieve. Twenty four soil mixtures were then prepared as shown in the following Table 2 using the 1 kilogram amounts of soil in the 4 liter capacity plastic bags. The test materials were added to the bags and mixed thoroughly with the moist (60% field capacity) soil and transferred to 1 liter capacity 4-inch diameter cylindrical plastic pots.

[0045] It can be seen from Table 2 that the maraschino and tart cherry pit meal were added to the soil at rates of 0, 2, 4, and 6 grams per kilogram of soil. These maraschino and tart cherry pit meal treatments were added alone and in combination with 2 and 4 grams of commercially available soybean meal per kilogram of soil. Thus, there were a total of 24 treatments in the experiment of Example 2. The 24 treatments represented all rates of soybean meal and maraschino and tart cherry pit meal alone and all possible combinations of soybean meal and maraschino and tart cherry pit meal rates. Each rate and controls were represented by eight replications (experimental unit = one pot) arranged in a randomized complete block design in a greenhouse. A no-treatment control and a positive control consisting of 10 milliliters of 10% metam sodium (Vapam™), a broad-spectrum fumigant, were included in each experiment.

TABLE 2

Mixture Number (8 replications)	Soil (kg.)	Tart Cherry Pit Meal (grams)	Maraschino Cherry Pit Meal (grams)	Soybean Meal (grams)	Vapam™ 10% sodium N-methyl dithiocarbamate (ml.)
No Treatment Control	1	0	-	0	-
Positive Control	1	0	-	0	10
2-1	1	0	-	0	-
2-2	1	2	-	0	-
2-3	1	4	-	0	-
2-4	1	6	-	0	-
2-5	1	0	-	2	-
2-6	1	2	-	2	-
2-7	1	4	-	2	-
2-8	1	6	-	2	-
2-9	1	0	-	4	-
2-10	1	2	-	4	-
2-11	1	4	-	4	-
2-12	1	6	-	4	-
2-13	1	-	0	0	-
2-14	1	-	2	0	-
2-15	1	-	4	0	-
2-16	1	-	6	0	-
2-17	1	-	0	2	-
2-18	1	-	2	2	-
2-19	1	-	4	2	-
2-20	1	-	6	2	-
2-21	1	-	0	4	-
2-22	1	-	2	4	-
2-23	1	-	4	4	-
2-24	1	-	6	4	-

[0046] The pots were placed on a greenhouse table and were kept moist for 10 days when soil samples were collected from each pot for nematological analysis using methods described in Rodriquez-Kabana *et al.*, "A simple method for extraction nematodes from the soil", *Nematropica* 11: 175-185, 1981. The pots were then planted (5 seeds/pot) with "Rutger" tomato. The ensuing plants were kept in good growing conditions for eight weeks when the plants were removed

and soil samples collected for nematological analysis. The roots were examined for damage, the number of root galls caused by *M. incognita* were counted, and the weights of fresh roots and shoots recorded. The general appearance of the roots was assessed using a subjective index scale where 5 represented the worst looking roots and 1 those with a healthy appearance. Root condition was used as an indication of resistance to both nematodes and phytopathogenic fungi. The roots were incubated for 72 hours to determine nematode populations in their tissues using the same method as for the soil samples. All data were analyzed by standard procedures for analysis of variance and Fisher's Least Significant Differences were calculated ($P = 0.05$) when F values were significant. All differences among means referred to herein were significant at $P \leq 0.05$.

[0047] Several formulations evidenced considerable nematicidal activity. The addition of soybean meal provided for consistent activity against root-knot nematode. Application of the meals alone was less effective for complete root-knot nematode control. Mixtures containing 4 grams soybean meal + 6-8 grams cherry pit meal resulted in the tallest, heaviest, and most vigorous plants with the best looking root systems and the lowest numbers of root-galls caused by *M. incognita* (>80% reduction in root galls). These mixtures were superior to Vapam™ in plant response and approach the fumigant in nematode control. There was a slight advantage to using tart cherry pit meal over the maraschino cherry pit meal for nematode control.

<u>Example 3</u>

[0048] An extract of cherry pits was made by combining 100 grams of ground cherry pit meal with 200 milliliters of ethanolamine. This was mixed thoroughly and heated in a microwave on high for 3 minutes to achieve the boiling point of the ethanolamine. This solution was mixed well and combined with 300 milliliters deionized water. Following mixing the solution was passed through a glass wool filter in a buchner funnel. Approximately 400 milliliters of liquid extract was obtained. Cherry pit extract was also prepared using ethanolamine or hexane as the solvent in a Soxhlet extraction.

[0049] Extracts were analyzed for amygdalin content using the following method. The equipment consisted of a Perkin Elmer PE series 200 LC pump with

a LC 295 UV/Vis detector. The system is equipped with an Alcott 728 autosampler using a 20ul injection loop. The column and guard column used were from Alltech; Alltima C18 5u, (250x 4.6 mm.) and Alltima C18 5u (7.5x 4.6 mm.). The detector was set for 210 nanometers. The flow rate of the mobile phase was set for 1.0 ml./min. The column was kept at room temperature. The gradient consisted of a mixture of acetylnitrile and water over a period of sixty minutes. Both standards and samples were diluted with a mixture of 70% acetylnitrile and 30% water. Samples were passed through a 0.45µm filter prior to analysis. A standard curve for amygdalin (100-400 ppm) was generated using the 60 minute gradient method. Standards were prepared in 100 milliliter volumetrics with the 70% solution of acetylnitrile.

[0050] Soil for the experiments of Example 3 was obtained from a cotton field infested with root-knot nematode (*Meloidogyne incognita*). The moist (approx. 60% field capacity) soil was screened (1 mm. mesh) and mixed 50:50 (v:v) with moist washed siliceous river sand (1 mm. mesh); the mixture, henceforth referred to in Example 3 as soil, was apportioned in one kilogram amounts contained in 4 liter capacity polyethylene bags. The requisite materials were added to each bag and mixed thoroughly. The contents of the bags were then transferred to 1 liter cylindrical PVC pots. The pots were placed in a greenhouse and kept moist for 2 weeks when soil samples were collected to determine nematode populations and each pot planted with a single 3 week old "Rutger" tomato seedling. The plants were kept in optimal growing conditions for 6 weeks and were then removed from the pots and washed to remove soil from the roots. Soil samples were taken from each pot after removal of the plants. Data was collected on plant height, the weight of shoots and roots, and the number of root galls caused by *M. incognita*. Nematode populations in roots and soil samples were determined using the method of Rodriquez-Kabana *et al.* used in Example 1 and 2. This extract was also added to nematode and fungi infected soil as a pre-plant treatment, at varying concentrations, for the growth of yellow straight-neck squash. Experimental details were identical to those for tomatoes.

[0051] For each type of cherry (maraschino and tart), two types of extracts were tested as described above (one with microwave extraction and the other by

Soxhlet). Experiments were conducted following the procedures described for Examples 1 and 2 and there was an experiment for each of the two types of cherries. In each experiment, the extracts were added at rates of 0, 2, 4, 6, 8, and 10 milliliters per kilogram of soil. No-treatment controls and positive controls with 10% aqueous Vapam™ sodium N-methyl dithiocarbamate at 5 and 10 milliliters per kilogram soil were included in each test. Data were analyzed following standard procedures for analyses of variance. Fisher's Least Significant Differences (FLSD) were calculated when F values were significant and are included in the graphs. Unless otherwise indicated all differences referred to in the text were significant at the 5% or lower level of probability.

[0052] HPLC analysis of the extracts showed that amygdalin was present in all extracts at concentrations of approximately 3,000-4,000 ppm. Potential solvents for extraction include ethanolamine, hexane, chloroform, super critical CO_2, propane, liquid nitrogen and mixtures thereof. Application of either tart or maraschino extract to soil effectively controlled root-knot nematode and promoted tomato growth and plant vigor (see Table 3). The plant response and nematicidal activity was equivalent or better than observed in plants from soils treated with 10% aqueous Vapam™ sodium N-methyl dithiocarbamate. Soxhlet extract was only slightly less effective than the microwave procedure for preparation of nematicidal extracts. The tart cherry microwave extract was effective against *M. incognita* at rates $\geq$ 2 milliliters per kilogram of soil but the Soxhlet required $\geq$ 4 milliliters per kilogram of soil. The tart cherry extract was equivalent to the maraschino preparation for root-knot nematode control.

[0053] This extract was also added to nematode and fungi infected soil as a pre-plant treatment, at varying concentrations, for the growth of yellow straight-neck squash. The extract was effective at controlling root-knot nematodes, stunt nematodes, and lance nematodes at applications as low as 2 milliliters per kilogram of soil. This soil was used to plant tomatoes two months after planting of the squash, and one week following harvesting of the squash, without further treatment. The extract remained active and completely eliminated nematode populations during the growth of the tomatoes, while stimulating the population of saprophagous nematodes (>300% increase). Shoot and root weights increased

>200% over controls and overall root condition was excellent, indicating resistance to both nematode root gall and phytopathogenic fungi. It is clear that that an extract of cherry pits is efficacious in controlling nematodes and promoting overall good plant health in squash and tomatoes.

Table 1. Effect of Maraschino CPM Extract (Microwave) on Nematodes

Treatment (ml. Extract / kg. soil)	Root-knot larvae per 100 cm^3 of soil	Saprophagous nematodes per 100 cm^3 of soil
0	781.1	66.6
2	294.3	222.1
4	9.9	349.3
6	0.0	194.4
8	0.0	293.3
10	0.0	216.1
10% Vapam™ (5 ml)	0.0	0.0

[0054] Therefore, it can be seen that there has been provided inexpensive, non-phytotoxic nematicidal composition that can be substituted for environmentally hazardous and toxic synthetic nematicides. The organic amendment controls nematodes and other crop pests. A method of producing the amendment and application of the amendment to soils containing growing plants has also been provided. The amendment effectively controls several types of phytopathogenic nematodes and fungi with no observed phytotoxic effects. The invention provides nematicidal compositions and methods for reclaiming and managing nematode-infested soils, for stimulating the growth of plants in nematode-infested soils, and for imparting sustained nematicidal and fertilizer activity to the soils. The invention also provides soil amendments for the control of phytopathogenic nematodes, and methods for producing the soil amendments. The invention further provides compositions which are obtained from organic sources and which stimulate the reproduction and development in soil of normal microflora which are destructive to plant-pathogenic nematodes.

[0055] Although the present invention has been described with reference to certain embodiments, one skilled in the art will appreciate that the present invention can be practiced by other than the described embodiments, which have been presented for the purpose of illustration and not of limitation. Therefore the

scope of the appended claims should not be limited to the description of the embodiments contained herein.

INDUSTRIAL APPLICABILITY

[0056] The present invention relates to nematicidal compositions and soil amendments for the control of phytopathogenic nematodes. The invention also provides methods for reclaiming and managing nematode-infested soils, for stimulating the growth of plants in nematode-infested soils, and for imparting sustained nematicidal and fertilizer activity to the soils.

CLAIMS

What Is Claimed Is:

1. A method for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium, the method comprising:

applying to the plant growth medium at least a nematistatically and fungistatically effective amount of ground seeds from a drupe of the *Rosaceae* family.

2. The method of claim 1 wherein the drupe is selected from the group consisting of cherry, peach, apple, pear, quince, almond, plum, and apricot.

3. The method of claim 1 wherein the drupe is selected from any plant of the genus *Prunus* of the *Rosaceae* family.

4. The method of claim 1 wherein the plant growth medium is soil and the amount of ground seeds is at least 0.5 grams per kilogram of soil.

5. The method of claim 1 wherein the plant growth medium is soil and the amount of ground seeds is about 0.5 to about 10 grams per kilogram of soil.

6. The method of claim 1 further comprising applying to the plant growth medium a meal other than the ground seeds.

7. The method of claim 6 wherein the meal is selected from the group consisting of soybean meal, cottonseed meal, sunflower seed meal, linseed meal, peanut meal, safflower meal, corn meal, jojoba meal, and sesame meal.

8. The method of claim 6 wherein the meal is soybean meal.

9. The method of claim 1 further comprising applying to the plant growth medium urea or a source of urea.

10. The method of claim 1 wherein the ground seeds pass a 0.1 millimeter sieve.

11. A method for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium, the method comprising:

applying to the plant growth medium at least a nematistatically and fungistatically effective amount of an extract of seeds from a drupe of the Rosaceae family.

12. The method of claim 11 wherein the drupe is selected from the group consisting of cherry, peach, apple, pear, quince, almond, plum, and apricot.

13. The method of claim 11 wherein the drupe is selected from any plant of the genus *Prunus* of the *Rosaceae* family.

14. The method of claim 11 wherein the plant growth medium is soil and the amount of extract is at least 0.5 milliliters per kilogram of soil.

15. The method of claim 11 wherein the plant growth medium is soil and the amount of extract is about 0.5 to about 10 milliliters per kilogram of soil.

16. The method of claim 11 wherein the extract is prepared grinding the seeds and extracting the seeds with a solvent selected from the group consisting of ethanolamine, hexane, chloroform, supercritical carbon dioxide, propane, liquid nitrogen, and mixtures thereof.

17. The method of claim 16 wherein:

the seeds are from any plant of the genus *Prunus* of the *Rosaceae* family,

the solvent is ethanolamine, and

a mixture of the seeds and the solvent is heated during extraction to temperatures of 20°C to 75°C.

18. The method of claim 16 wherein:

the seeds are from any plant of the genus *Prunus* of the *Rosaceae* family,

the solvent is hexane, and

a mixture of the seeds and the solvent is extracted using soxhlet extraction.

19. The method of claim 11 wherein the plant growth medium is a plant growth solution.

20. The method of claim 11 wherein the growth of saprophagous nematodes is stimulated.

21. A method for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium, the method comprising:

applying to the plant growth medium at least a nematistatically and fungistatically effective amount of a material including amygdalin.

22. The method of claim 21 wherein the material is a ground solid material.

23. The method of claim 21 wherein the material is a solution.

24. The method of claim 21 wherein the amygdalin is present in the material in an amount of at least 100 ppm of the total weight of the material.

25. The method of claim 21 wherein the amygdalin is present in the material in an amount of 100 ppm to 30,000 ppm of the total weight of the material.

26. A composition for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium, the composition comprising:

ground seeds from a drupe of the *Rosaceae* family; and

a meal selected from the group consisting of soybean meal, cottonseed meal, sunflower seed meal, linseed meal, peanut meal, safflower meal, corn meal, jojoba meal, and sesame meal.

27. The composition of claim 26 wherein the drupe is selected from the group consisting of cherry, peach, apple, pear, quince, almond, plum, and apricot.

28. The composition of claim 26 wherein the meal is soybean meal.

29. The composition of claim 28 wherein the weight ratio of ground seeds to soybean meal is from 0.25 to 5.

30. The composition of claim 26 wherein the drupe is selected from any plant of the genus *Prunus* of the *Rosaceae* family.

31. The composition of claim 30 wherein the meal is soybean meal.

32. The composition of claim 21 wherein the weight ratio of ground seeds to soybean meal is from 0.25 to 5.

33. The composition of claim 26 further comprising:

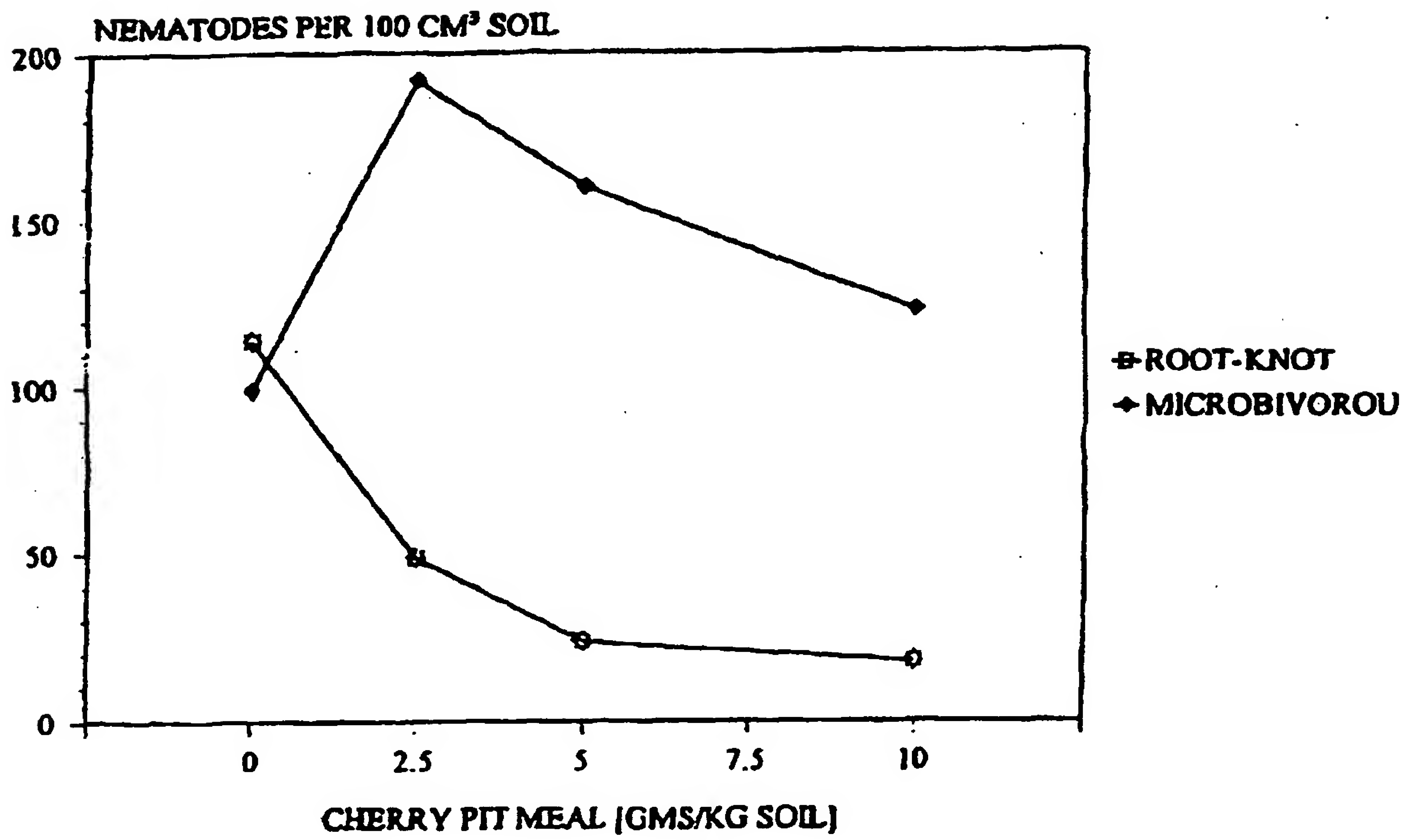
urea or a source of urea.

34. The composition of claim 33 wherein the weight ratio of ground seeds to urea is from 0.01 to 6.

35. The composition of claim 33 wherein the meal is soybean meal.

36. The composition of claim 21 wherein the ground seeds pass a 0.1 millimeter sieve.

Figure 1


NEMATODES PER 100 CM³ SOIL
200
150
100
50
0
0
2.5
5
7.5
10
CHERRY PIT MEAL (GMS/KG SOIL)
ROOT-KNOT
MICROBIVOROU

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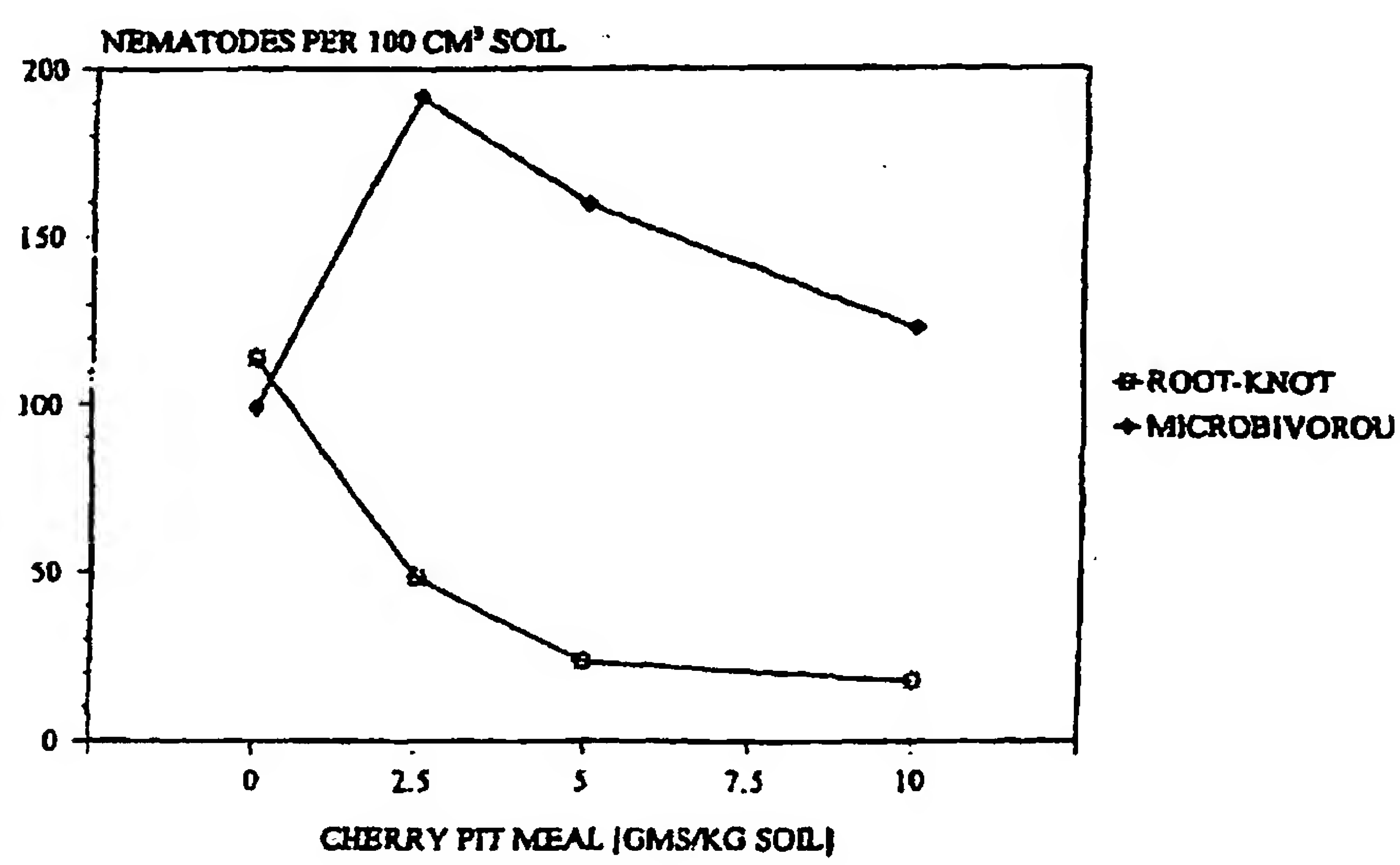
[Continued on next page]

(54) Title: THE USE OF DRUPES AS A SOIL AMENDMENT TO CONTROL NEMATODES



(57) Abstract: A method for suppressing the growth of phytopathogenic nematodes and fungi in a plant growth medium, and an inexpensive, non-phytotoxic nematicidal composition that can be substituted for environmentally hazardous and toxic synthetic nematicides is disclosed. The composition comprises the ground seeds from a drupe of the*Rosaceae* family (e.g., cherry, peach, apple, pear, quince, almond, plum, and apricot), or an extract of these ground seeds. The composition may also include a metal such as soybean meal, and urea or a source of urea. The composition is applied to a plant growth medium in at least nematistically and fungistatically effective amount in order to suppress the growth of phytopathogenic nematodes and fungi in the plant growth medium.

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US CL :424/ 405, 725, 735, 765, 777; 435/242, 325, 410, 418
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B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,207,705 B1 (COATS et al) 27 March 2001, see the entire document and specifically note col. 2 and 17, all lines.	1-36
A,P	US 6,310,273 B1 (GILCHRIST et al) 30 October 2001, see the entire document.	1-36
A	US 5,859,235 A (LIU et al) 12 January 1999, see the abstract and the col. 20, all lines.	1-36
A	US 5,792,467 A (EMERSON et al) 11 August 1998, see the entire document.	1-36

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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